REMARKS

1. STATUS INFORMATION:

Claims 1, 2 and 4-48 are pending and were examined. Claims 49-92 were previously withdrawn as being directed to a non-elected invention. Claim 3 was withdrawn as being directed to a non-elected species. Claims 1, 10, 34, 37 and 43 are hereby amended.

2. SUPPORT FOR THE AMENDMENTS TO THE CLAIMS:

The amendments made to claim 1 clarify initially confusing language. No new matter has been added, and the amended claim is supported by original claim 1 and by the specification as a whole. The limitation (a) in claim 1 is supported throughout the subject specification. See, for example the initial paragraph of the Summary of the Invention section (particularly, page 4, lines 24-27, underlining added):

> *The methods exploit the discovery that recombination, particularly recombinase recombination can occur between two or more constructs of the same type (e.g. having the same origin of replication and/or the same regulatory or selectable markers).*

The amendments to claims 10, 34, 37 and 43 are editorial in nature, merely correcting typographical error or introducing separation between individual steps as required by the Examiner.

3. CLAIM OBJECTIONS:

Claims 1, 2 and 4-48 were objected to under 37 CFR 1.75(i) for being presented in improper format. Applicants are grateful to the Examiner for having pointed this matter out, and have amended claim 1, as well as claims 10, 34 and 37 in order to separate different steps. The claims as amended are now in proper form, and the Examiner is kindly requested to withdraw this objection.

4. REJECTIONS UNDER 35 USC 112, 2nd PARAGRAPH:

Claims 1, 2 and 4-48 were rejected under 35 USC 112, second paragraph, as being indefinite. In particular, the Examiner notes various confusing or otherwise unclear phrases in claim 1. Applicants are in agreement with the Examiner, and have therefore amended claim 1 for clarity.

Additionally, Claim 45 was found indefinite in its recitation of the phrase "because mutations". Claim 45 is cancelled in this paper.

5. REJECTIONS UNDER 35 USC 102(b) – Johnson et al.

Claims 1, 2 and 4-48 were rejected under 35 USC 102(b) as being anticipated by Johnson et al., 2001. Applicants have studied Johnson et al. in detail. Johnson et al. is directed exclusively to "two-vector" recombination systems, wherein two different vectors, having different origins of replication, are used to achieve recombination.

The subject invention is directed to a "single vector" recombination system, wherein a single vector carries the variable sequences which are to be recombined. This

represents a fundamental difference over the prior art, in general, and over Johnson et al. in particular.

More specifically, Johnson et al. requires the use of two different vectors. See, for example, Johnson et al. column 12, lines 39-41:

> *The first vectors may be phages or phagemids and the second vectors plasmids, or the first vectors may be plasmids and the second vectors phages or phagemids.*

That Johnson et al. is only directed to "two-vector" recombination methods is consistently made clear throughout its specification. See, further, column 10, lines 28-43 and column 12, line 54 through column 13, line 2; most notably, the disclosure from line 67 of column 12 through line 2 of column 13 (*"said population of said second polypeptide chains not being expressed from the same vector as said population of first polypeptide chains"*). In addition, see column 14, lines 52-53 (*"The genes for both subunits present on two separate replicons..."*), and column 17, lines 29-30 (*"the concept of using two or more replicons to generate diversity..."*).

Similarly, Fig. 2 of Johnson et al. shows a recombination scheme using distinct phage and phagemid libraries. Fig. 3A shows a recombination scheme using distinct phagemid and plasmid libraries, wherein two different origins of replication are indicated.

There is no disclosure in Johnson directed to a "single-vector" approach to recombination.

As explained in the subject application (page 3, lines 12-30), the state of the prior art was essentially as follows:

> *The introduction of the different expression vectors carrying the genes to be recombined within the cell, however, posed serious problems to the*

> *creation of large diverse libraries. Typically, the two vectors had to be different (e.g. one vector a plasmid, the other vector a phage). This is because it had been reported that bacteria infected by a filamentous phage are resistant to further infection by other filamentous phage (Boeke et al. (1982) Mol Gen Genet 186: 185-192). One of the two vectors thus had to be subject to transfection rather than infection and this drastically lowered the efficiency of transformation of the cell.*
>
> *In addition, it was believed that the two constructs required different origins of replication belonging to different incompatibility groups and also different antibiotic resistance genes. This is because it was believed by experts in the field that plasmids that utilize the same replication system (origin of replication) cannot co-exist stably in a cell are said to be incompatible (see pages 1.3-4 of (Sambrook et al. (1989) Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press)). For each plasmid and origin of replication, a different antibiotic resistance gene was also required. This limited the total number of different plasmids which could be maintained within a single cell. Consequently, the number of recombination substrates that could be present within a single cell was limited to the number of different origins of replication and antibiotic resistance genes that could be used. Thus, the size and diversity of a library that could be made using this approach was seriously limited.*

The subject invention overcomes the various limitations of all two-vector approaches in the prior art, including the two-vector approach described by Johnson et al. The invention was, in part, the result of the unexpected discovery that recombination could occur between sequences located on the same type of vector. See Example 1 in the specification, beginning on page 41, as well as page 21, lines 1-8, reproduced in part below:

> *It was surprising discovery of this invention that, contrary to the teachings in the prior art, multiple constructs (vectors) of the same class (phagemid, plasmid, etc.) that are essentially identical in regulatory elements (e.g., origin and/or promoters and/or enhancers and/or termination sequences) and/or selectable markers (e.g., antibiotic resistance genes) can co-exist in a host cell (e.g. bacterial cell) for a sufficiently long time that extensive recombination ... can occur...*

Before the invention, the prior art taught that vectors having different origins of replication were required in order to achieve recombination of sequences resident on

two different vectors, and not between sequences resident on the same vector. The invention provided the art, for the first time, with the knowledge that vectors having the same origin of replication could facilitate recombination between sequences on different vectors.

In one embodiment described in the specification of the subject application (see FIG. 1), identical phagemids containing identical origins of replication are used to achieve recombination between antibody variable heavy and variable light chain genes. The experiments described in Examples 1 and 2 (beginning on page 41 of the specification) showed that at least five different phagemid could enter a single bacteria, and that two phagemid that entered a single bacteria were able to recombine with each other to equilibrium, resulting in four different phagemid. Example 3, beginning on page 46 of the specification, describes the successful use of identical phagemids to achieve extensive recombination of antibody variable heavy and light chain genes to generate a highly diverse scFv antibody library.

In conclusion, the following limitation included in step (i) of claim 1,

> *... wherein the members of the initial population of nucleic acid molecules, (i) have the same origin of replication...*

is simply not disclosed in Johnson et al.

6. CONCLUSION:

Applicants have amended the claims for clarity, and have addressed each of the Examiner's objections to and rejections. In addition, the foregoing discussion explains the fundamental distinction between the claimed methods (single-vector approach) and the cited prior art (two-vector approach). The claims are believed to be in condition for allowance. If in any respect the Examiner would require further clarifying amendments to any of the claims, a telephonic interview is kindly requested for expediency.

Respectfully submitted,

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